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LC World Talk

SHIMADZU'S NEWSLETTER FOR THE HPLC GLOBAL COMMUNITY

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Development of a Parallel LC/MS System for Quantitative ADME Analysis

Discover a unique valve switching and control system for dual-column LC/MS methodology that was developed to improve automation and throughput for ADME studies.

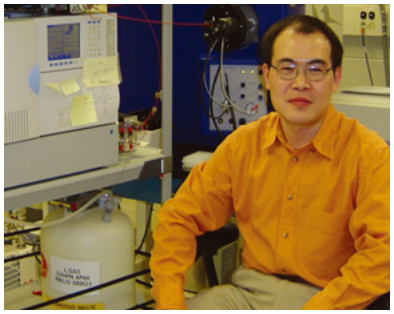


A unique valve switching and control system for dual-column LC/MS methodology was developed to improve automation and throughput for ADME studies. By implementing customer-modified dual-injection sequence on Shimadzu's SIL-HT auto-sampler, the consistency of sample analysis was greatly improved. The application of the parallel LC/MS methods for metabolic stability studies was also demonstrated.

DEVELOPMENT OF

Parallel LC/MS System FOR Quantitative ADME Analysis

by Lan Gao, Xueheng Cheng, Mark Schurdak, Lawrence Vernetti, Ken Matuszak, David Burns Global Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064



Lan Gao received his MS degree from West Virginia University and is currently a scientist at Abbott Laboratories. He has been developing analytical methodologies (HPLC, LC/MS) to support drug screening (lead compound and target validation) and lead compound optimization by analyzing chemical and biological substances using mass spectrometric methods, and those MS methods coupled with chromatographic techniques. He and his colleagues at Abbott were instrumental in developing the dual-column system and collaborating with Shimadzu. Lan has also received the prestigious GPRD Advanced Technology "Impact, Innovation, Integration" Award at Abbott in 2002.

ADVANCES IN HIGH-THROUGHPUT SCREENING have permitted the identification of large numbers of biologically potent and selective compounds worthy of a medicinal chemistry effort. However, the fact that a compound is active does not necessarily make it an attractive drug development candidate. It has been recognized that pharmacokinetic studies that assess compound absorption, distribution, metabolism, and elimination (ADME) should be initiated as early as possible in the drug discovery process to maximize the likelihood of development success and to minimize the costs associated with drug development. One of the challenges has been to develop reliable high-throughput analytical methods to evaluate the ADME properties for a large number of compounds.

Method

We have developed a parallel HPLC/MS system to increase the speed of compound quantitation. The system consists of one Shimadzu SIL-HT controller/autoinjector, four Shimadzu LC-10AVP pumps, and a series of switching valves. The firmware from Shimadzu SIL-HT has been customer-modified to implement a dual sample sequence for injection, and to control four LC pumps in a dual-binary format. Two identical analytical columns are used and a series of switching valves are controlled through a customized program using the Shimadzu SIL-HT system to direct the flow path. A Sciex Pulsar mass spectrometer is used for ESI-MS analysis. By staggering the acquisition time window and column auto-injector system equilibration, we have doubled the throughput of sample analysis.

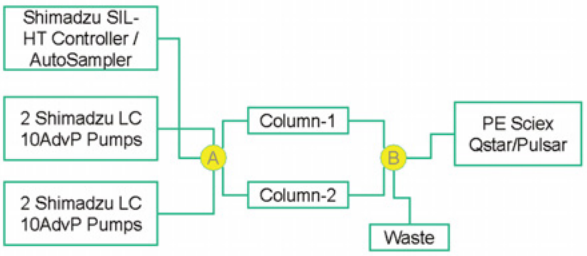


Figure 1—All valves and sample injections are controlled by Shimadzu's SIL-HT. 2-way communications were set up for the Shimadzu autosampler and Sciex mass spectrometer to synchronize sample injection and data acquisition—A, B switch valve respectively.

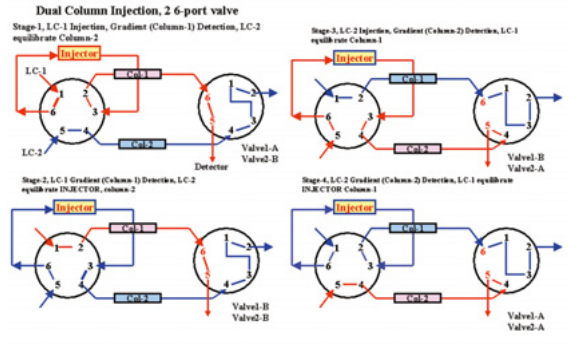


Figure 2

Experimental

All valves and sample injections are controlled by Shimadzu's SIL-HT. 2-way communications were set up for the Shimadzu autosampler and Sciex mass spectrometer to synchronize sample injection and data acquisition–A, B switch valve respectively.

Dual-Injection Table/Sequence

Dual-injection sequence was implemented on a Shimadzu SIL-HT system. The autosampler injects samples alternatively from the primary/secondary sample sequence table. This has been seamlessly coupled to the parallel LC/MS system we developed. By alternating between 2 different injection sequences, we can handle two different batches of samples simultaneously. The samples from the same batch are always analyzed on the same HPLC column as seen below in *Figure 3*.

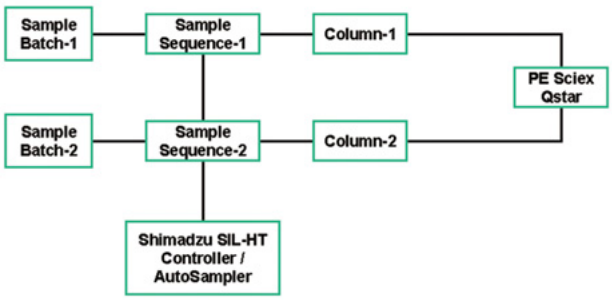


Figure 3

Switching Valve for Parallel HPLC

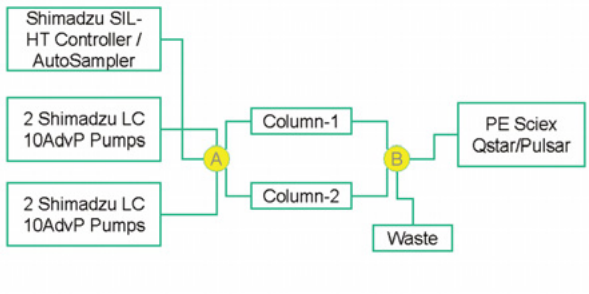


Figure 4—Valve configuration in the Scheme A(0)B(1)C(0) Injection to column-1, A(0)B(1)C(0) Injection to column-2, A(1)B(0)C(0)

Graphical Image of Timed Events for the Dual Column System

It is much easier to view graphically the time programs that are entered into the system controller instead of listing all the functions one by one. As can be seen in the following figures, two independent gradients are running in this system with re-equilibration of one column while the other column is performing sample analysis

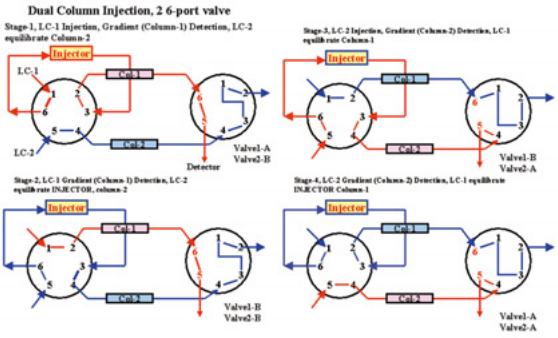


Figure 5— HPLC Column
Upchurch C18 guard column, 5 um, 1x10mm

Mobile Phase
A: H2O with 0.01% formic acid
B: Acetonitrile with 0.01% formic acid

PROCEDURES

A mixture of compounds containing internal standards was incubated with rat liver microsomes at 1 mg/ml concentration in the presence of 1 mM NADPH in Hanks balanced saline solution. After a one-hour incubation, ZnSO4 was added to a final concentration of 250mM to stop the reaction and precipitate the protein.

The samples were centrifuged and the supernatant was transferred into 96-well plates for LC/MS analysis. Two or four batches of samples were analyzed simultaneously using the parallel LC/MS method developed. After LC/MS analysis, quantitation working curves were constructed for every compound. For blank control, the same mixture of compounds was incubated with rat liver microsomes in the absence of NADPH. The ratio of remaining compounds in the presence and absence of NADPH was used to obtain the metabolic stability.

The analysis results are in the facing chromatograms and graphs.

Conclusions

- By using dual-injection sequences, samples from the same batch could be analyzed on the same HPLC column. Thus, variation from the use of different columns is minimized.
- By staggering the LC data acquisition time with column/autosampler equilibration time, the throughput of sample analysis has been increased by about 70%.

Acknowledgements

Thanks to Shimadzu Scientific Instruments for the customer support and travel support to the ASMS conference.

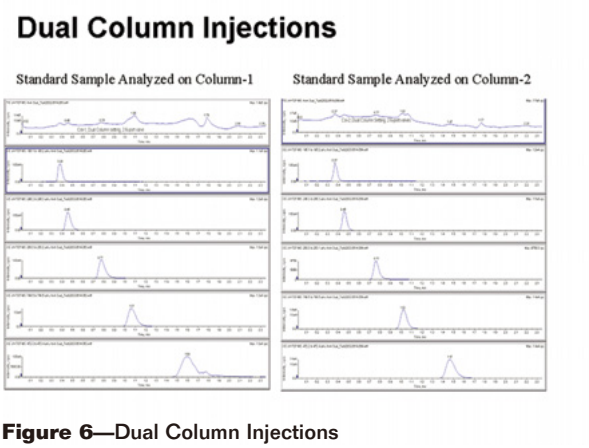


Figure 6—Dual Column Injections

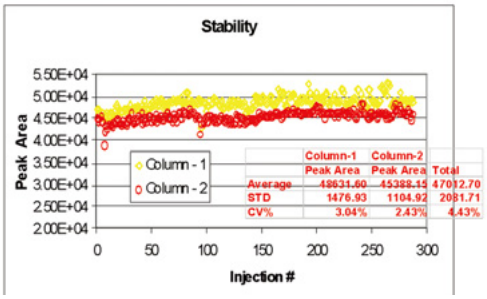


Figure 7—Samples were spiked into rat plasma to check the system stability.

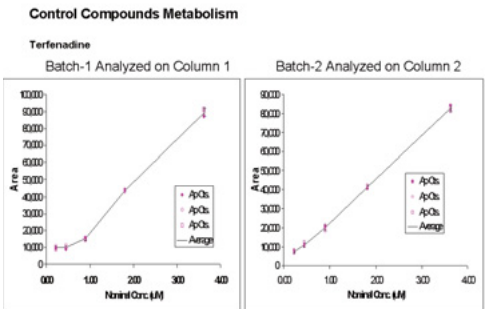


Figure 8

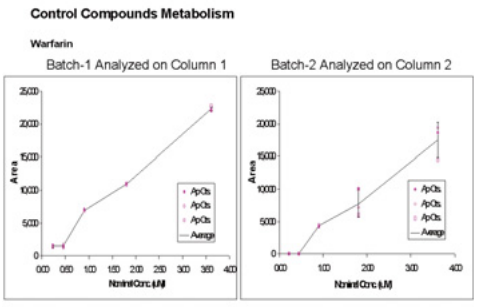


Figure 9

SHIMADZU'S SOFTWARE DEVELOPMENT GROUP collaborates with process chromatography chemists to design a user interface and control software that dramatically reduces the time to run multiple samples on numerous columns with varied mobile phases

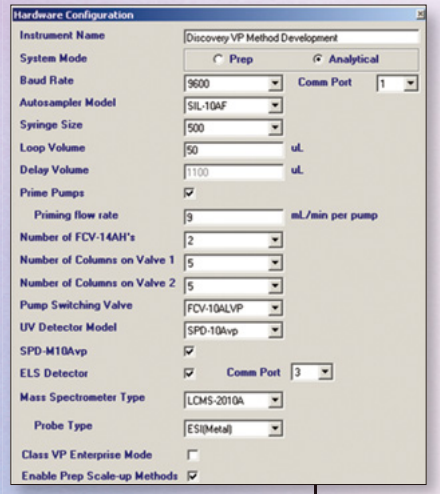
THE DRUG DEVELOPMENT PROCESS in the pharmaceutical industry is often pictured as a funnel. At the top, a vast number of compounds synthesized by combinatorial chemistry make their way through high-throughput screening to lead generation and optimization, on to metabolism, toxicology, and formulation studies, and finally to clinical trials and production. Method development is a critical part of this process. Researchers need to optimize separations quickly to send drug candidates on their way to scaleup and further evaluation.

Automated Method Development Software for Open Access Operation

In past LC WorldTalk articles, Shimadzu introduced Discovery VP for automated analytical and preparative HPLC for the discovery chemist. These systems pioneered the drug discovery arena and allowed medicinal chemists to concentrate on the chemistry instead of the chromatography. Now Shimadzu has further refined the software application to automate method development. This new tool can be applied not only in the area of drug development, but also in most applications in high performance liquid chromatography, thereby automating method development.

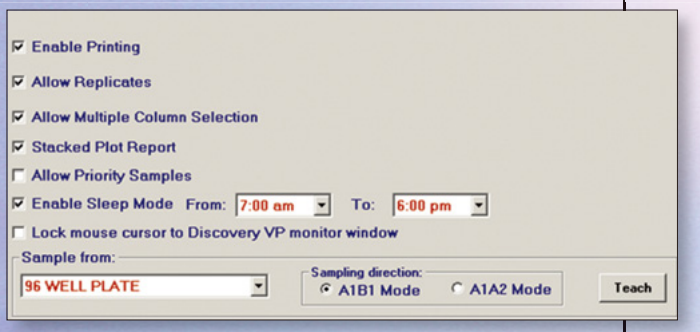
Method development has traditionally been very time-consuming, requiring expertise in column selection, and "trial and error" gradient analyses to determine the optimum solvent system and gradient profile for separations. In an effort to streamline the method development process, Shimadzu's software development group collaborated with process chromatography chemists at one of our major pharmaceutical customers to design a user interface and control software that would dramatically reduce the time it takes to run multiple samples on numerous columns with varied mobile phases.

All laboratories have users with a wide variety of HPLC experience, so an important goal of the software development was to design a program that is easy to learn and use, while keeping the necessary functions to produce meaningful data.



System Configuration

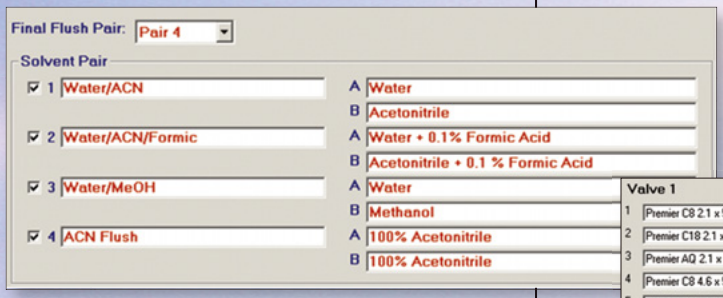
A System Administrator is responsible for configuring the hardware present on the system: autosampler, solvent valve, and detector types are entered in the Hardware Configuration screen. Autosampler choices include the fixed-loop style SIL-10AF, semi-micro SIL-10ADvp, and the high-speed, high-capacity SIL-HT. Solvent selection valves accommodate two or four solvents, and up to ten user-defined columns can be configured. Detector choices include UV, PDA, ELSD, MS, or any "other" detector with an analog output.



Preferences

In the Preferences section of the software, the Administrator enters additional system parameters to enable printing, email notification, and number and identity of solvents and columns.

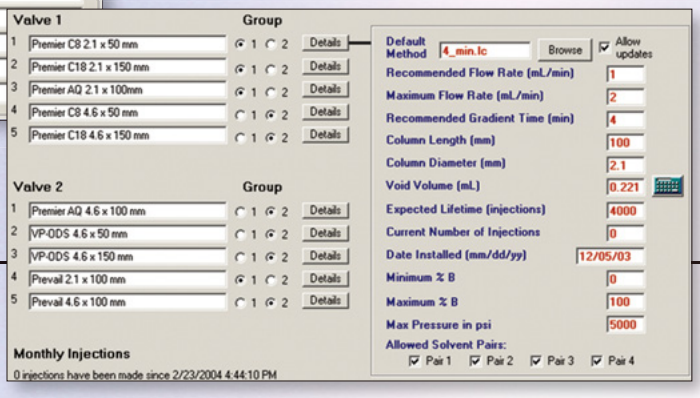
Discovery VP has a standby or "sleep" mode when submitted runs are complete. This conserves solvent but keeps the system available for immediate operation when the next user adds a new sample.



Solvent Pairs

Available solvent pairs are activated and described by the Administrator, and will appear on the printed run report. One pair may be assigned to flush all of the columns used in the queue when the system shuts down.

Also, the Administrator can restrict which solvents contact certain columns to prevent incompatibility with varied stationary phases.

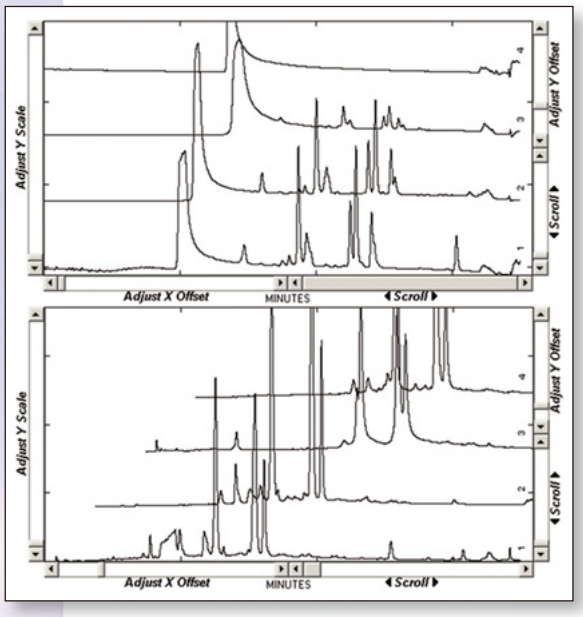


Columns

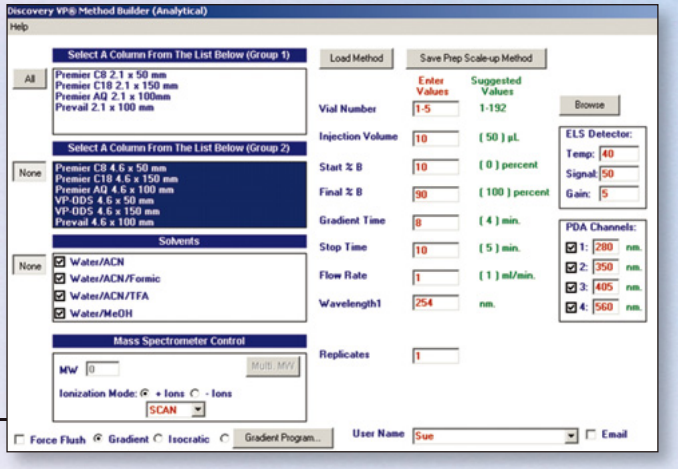
On the Columns tab, the Administrator supplies column information and sets limits for flow, pressure, and amount and type of solvent that can contact each column. Columns can be assigned to different groups based on size or chemistry. An entire group may be selected for analysis in the Method Builder screen.

Nutraceutical Example

A real-life example of the Method Development System was to investigate the separation of a milk thistle extract. The nutraceutical market has experienced a tremendous amount of growth in the last decade, and organizations like the Institute for Nutraceutical Advancement (http://www.nsf-ina.org/) are trying to establish methods for consistency in analyzing various plant and herbal raw materials and extracts.



This example is a milk thistle extract tablet run with a water/methanol gradient, then with a water/acetonitrile gradient. The report clearly shows that the ACN gradient gives a better chromatographic profile, and also identifies the columns with the best component resolution. Now the method can be scaled up and the sample can be run on a prep system to isolate and purify individual components.



Method Builder

When the Administrator completes the system configuration, Discovery VP is ready for walk-up sample submission. The Method Builder screen available to users is easy to navigate, letting analysts of all levels of HPLC experience run samples with a minimum of mouse clicks.

The Discovery VP Analytical System for Method Development can be configured with ten columns and four solvent pairs, so a chemist has the potential for 40 unique run conditions for each sample. Entire groups of columns can be selected to samples with one or more solvent pairs. The resulting chromatograms are displayed on a Stacked Plot Report and have multiple display options: zoom, baseline and X-Y axis offset, or isolating specific chromatograms for comparison.

Method Development for Everyone

Although the Discovery VP software was developed in conjunction with pharmaceutical scientists, it is not exclusive to that type of research environment. Any laboratory with multiple users, a wide variety of samples, and goals of method development and/or preparative scaleup and purification will benefit from a Discovery VP system.

Pharmaceutical Labs: Use Discovery VP preparative systems at the top of the drug discovery funnel for screening and isolating combinatorial products, then analytical systems for method development for isolating metabolites and impurities. For scaleup and active ingredient purification, preparative systems are again valuable.

Nutraceutical Companies: Raw material purification may start with kilograms of botanical material, and a preparative system will reliably isolate compounds from extractions of leaves, flowers, etc. Analytical systems determine the best separation conditions for isolating active ingredients, and ensure dosage accuracy from batch to batch of final product.

University Research Labs: As a shared resource for an entire department or a dedicated system for a synthetic group, a Discovery VP preparative system will simplify or eliminate flash chromatography. No more wasting time standing in front of a hood moving a rack of test tubes around to collect every drop from a flash column. Discovery VP will collect only peaks, and there are options to limit fraction volumes to ensure purity and isolate only the most concentrated fractions for use in subsequent reaction steps.

Throughout its development, customers contributed to both the system functionality and design of the user interface to give Discovery VP more flexibility and to make it even easier to use. All suggestions from customers are evaluated for possible inclusion in future software versions and dedicated programmers constantly troubleshoot and improve its features. The result is a powerful, easy-to-use software package that does the method development work for you.

LIQUID CHROMATOGRAPHY COMBINED WITH MASS SPECTROMETRY for detection has enabled many chemists to find and quantify components of interest from a variety of complicated matrices. The technique can speed up HPLC method development, improve quantitation, and confirm the identity of an unknown. When chromatographers are concerned about sensitivity and reproducibility, they often turn to information-rich detectors such as an LC/MS to improve sensitivity. This article goes over some of the LC/MS basics and provides some suggestions to help anyone become more successful with LC/MS.

Getting the Most from LC/MS

You only see the compounds that ionize!

Probably the most common issue is recognizing that target compounds must be ionized BEFORE they enter the LC/MS vacuum region. In general, the ionization processes for LC/MS are not very efficient. Often, only one ion out of many thousands or millions of molecules actually becomes a detection event. There are many ways in which ions can lose their charge, and only a limited number of ways to create a charged species in the first place.

Over the years, HPLC users have gotten used to a particular brand of HPLC column or a specific chemistry for the separation, and often try this when doing LC/MS. Getting the best separation usually involves operating at conditions that usually reduce ionization of target species (unless you are doing ion exchange chromatography). So when compounds are neutral in order to get a great separation, is it any wonder that signal intensity at the LC/MS detector might be a little low?

For example, when you want to analyze organic acids by HPLC, you usually need to suppress the ionization in order to obtain a good separation. This is generally done by adding a dilute acid to the mobile phase. To detect organic acids by LC/MS, you need to facilitate ionization. How to get both of these to work at the same time? Consider using additives that don't suppress all the ionization. Rather than add a lot of acid to the mobile phase to reduce ionization, it may be better to use a volatile ion pair reagent instead. So rather than picking a column, adjusting the mobile phase, and looking for a peak, do the decision process the other way around. Start by picking a chemistry that will enable your target compounds to produce ions, then find a separation mechanism that will allow for a separation with those conditions, then select the column type that is compatible.

Shown in *Figure 1* is a separation of normal physiological amino acids performed using a volatile ion pair reagent in the mobile phase. This allows the separation of the ion pairs as neutral species, so the separation can be performed using typical C-18 columns. When the compounds are sprayed into the ion source of the mass spectrometer, the ion pairs separate and you get a charged ion that can be easily detected by mass spectrometry. This approach eliminates the need for derivatization, and simplifies the method development considerably.

Obtaining the best sensitivity from LC/MS

One of the most significant advantages of LC/MS is the incredible sensitivity of the mass spectrometer. Detectable compounds can often be analyzed at the low picogram levels. However, many people indicate they have difficulty obtaining the same levels every day, usually resulting from two situations. One is a high background signal at the target m/z. This usually indicates that a large amount of the target has been run and is giving a high background signal, or that the mobile phase and/or instrument are contaminated with something that has the same mass. Simple cleaning with a suitable solvent usually helps, although a stronger solvent usually works better.

If you want to get the best signal-to-noise results, you also should pay attention to the cleanliness of the instrument, the containers to be used, the syringes for injection, and the way that everything is handled. Simple things like a trace PPB level of detergent or even part per billion levels of some metals can drastically affect proteins and peptides to the extent that peaks can disappear.

To minimize these problems, dedicate glassware specifically for LCMS use. Keeping impurities out of the system should be an important aspect of method development. For the mobile phase, use fresh solvent and clean water. Test these by running a mass spectral blank to see what impurities are already present. Water can differ daily. Even water in reverse osmosis systems can be contaminated with plasticizers, glycols and other compounds. Solvents should also be tested frequently. What starts off as good methanol or acetonitrile can become contaminated by organic impurities in air that dissolve in the organic solvent.

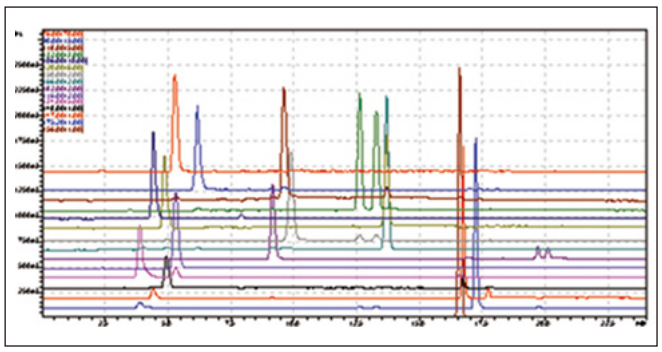


Figure 1—LCMS Analysis of Amino Acids using Ion Pair Reagent

Instrument: Shimadzu LCMS-2010A
Column: Shim-pack VP-ODS (2.0 mm I.D. x 150 mm L)
Mobile phase A: 5 mM ICP-MS7-water, Mobile phase B: acetonitrile
Gradient program: 15%B (0 min) => 35%B (12 - 25 min)
Ionization mode: ESI positive
CDL temp: 200C, BH temp: 200C, CDL voltage: 20 V, Q-array voltage: 15, 10, 5 V, Q-array RF: 120

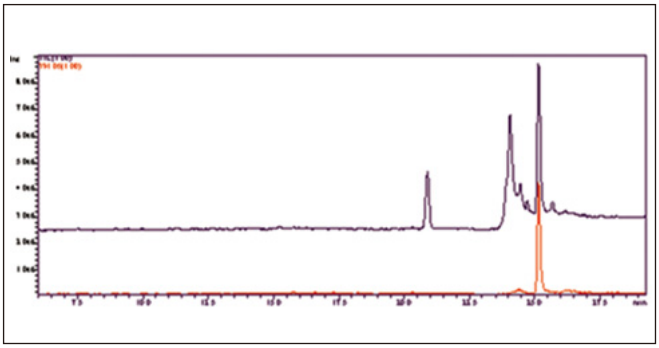


Figure 2—Analysis of Gingko extract.

Red trace shows contamination of mass chromatogram by diethylhexyl phthalate (m/z 391) dissolved in the 'B' solvent. Phthalate in this example came from label tape used near the solvent container.

What is a Picogram?

- Sensitivity of quadrupole LCMS ≤ 0.1 picograms
- Average amount of erythromycin in surface water (106 river streams according to USGS) = 1 picogram/microliter
- Weight of about 100 E. Coli bacteria cells = 3 picograms
- Total organic carbon in HPLC grade water = up to 50 pg/microliter
- EPA Arsenic in drinking water std = 55 picograms/microliter
- EPA Cyanide in drinking water std = 200 picograms/microliter
- One pollen grain (Alder tree) = 9,700 picograms
- Solubility of fused quartz in high purity 18 megohm water = 11,000 picograms/microliter/4 weeks

One typically messy fingerprint = 20,000,000,000 picograms

One common indicator that the organic phase is contaminated is the presence of an ion at 391 m/z in positive ion mode. This is usually due to dioctyl phthalate (DOP or diethylhexyl phthalate). No matter how careful you are, this will eventually show up at a chromatographic peak and produce a very strong ion either by electrospray or APCI.

Where does it come from? It would probably be better to ask the question the other way around – where doesn't it come from? Nearly everything in the lab from plastic tubing, jar seals, clothing (especially if you have been in a car recently), label tape and even floor tiles often contains significant levels of phthalates. These can have enough vapor pressure to get into the ambient air. Since these compounds are extremely soluble in methanol or acetonitrile, they will concentrate there after just a few days in even some of the cleanest laboratories. DOP ionizes quite easily and will cause a background signal in any mass spectrometer eventually. So keep your solvent containers covered or sparged, or else change solvent often to prevent the build-up.

Mechanism of Ion Formation

No discussion of LC/MS optimization would be complete without looking at how ions form in the common LCMS interfaces, and the conditions that affect the yields. The two most common ion sources are electrospray (ESI) and atmospheric pressure chemical ionization (APCI).

ESI is concentration dependent; narrow peaks give the best results in terms of signal to noise. Anything that improves peak shape will contribute to improved sensitivity, so low flow rates, high-efficiency columns, and short run times will all improve sensitivity. Solvation also plays an important role on ionization efficiency. Picking the solvent and pH that gives the highest solubility usually results in the best sensitivity. Surface tension affects ESI sensitivity – even at low levels, soap, salts and even volatile buffers at high concentrations can negatively affect sensitivity.

One of the most frequently used additives nearly always results in reduced signal levels. Trifluoroacetic acid can reduce signal levels for proteins and peptides dramatically through several different mechanisms.

If TFA must be used, adding a small amount of proprionic acid can sometimes restore some of the lost sensitivity.

To go low in sensitivity, try keeping the concentrations of additives low. It is not uncommon to find that a lower level of most additives can result in improved sensitivity. Often additives will work better at 0.1 M and lower concentrations than at 0.2 or 0.5 M. Also, to get the best results for ESI, keep the water content of the mobile phase low. High levels increase ion current flow at the probe, which can reduce signal, and high water levels can lead to baseline instability as well.

Likewise, APCI has some properties that improve performance. In general, column diameter, particle size and flow rate are not as critical for APCI as with ESI. This is because APCI does not ionize directly, but rather depends on a second order reaction step after ionization of the mobile phase and nebulizer gas. The final results will depend on gas phase reactions and charge transfer. To get the best results, it is usually better to inject a lower concentration over a wider peak than to try to make the sharpest peak.

With APCI in negative ion mode, the best results will occur if a small amount of air is present. Be careful not to get to a combustion supporting level, however. For negative ionization, oxygen (or chlorine) is necessary to create the ion. A tightly sealed source, high levels of nebulizer gas or drying gas, or too much degassing of the mobile phase can contribute to smaller signal levels. Adding a small amount of air by way of an oxygenated solvent that is not perfectly degassed will usually produce better results in negative ion mode.

Pick the Right Ion Source

If the goal is maximizing the probability of detecting the target compound on the first try, pick conditions that will produce the desired ion easily, and not complicate the interpretation.

Look at the analysis of taxol as an example. This is a compound that is known to easily turn into a sodium or potassium adduct in electrospray mode. Even adding high levels of acid modifiers doesn't help much. Too little and the sodium adduct will predominate, and too much will cause hydrolysis of the taxol to a much

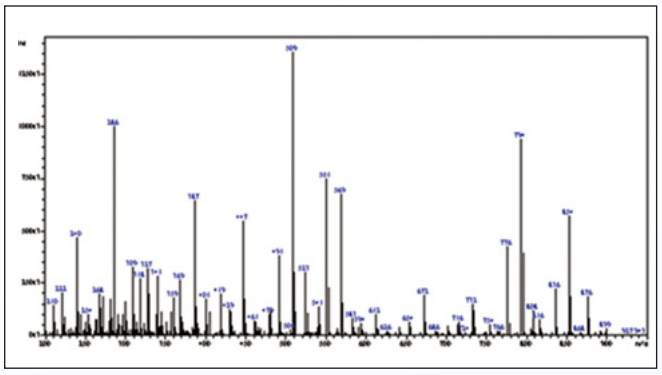


Figure 3—Paclitaxel Spectrum by APCI

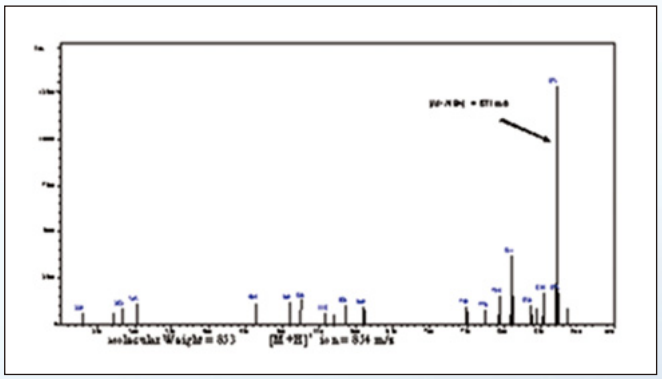


Figure 4—Paclitaxel Spectrum by APCI with NH4OAc

lower mass species. A better approach is to use APCI to produce the ions. APCI generally does not produce sodium adducts as easily as electrospray. In figures 3 and 4, taxol is shown analyzed by APCI. In the first case, the molecule has broken down due to acid conditions and temperature. In the second case, the addition of a small amount of ammonium acetate has stabilized the ion and allows for easy quantitation of taxol.

See Figures 3 and 4.

Pick the additives based on ionization benefits rather than separation aspects

There are two approaches to LCMS method development. If you are looking at maximizing qualitative information, you could start with picking the conditions that produce the best separation of the most compounds. This is a fairly traditional approach, and has been used in drug discovery for many years. Over the past few years, however, many pharmaceutical companies have started to look at the problem as one of detection rather than separation. By using shorter columns, faster flow rates, and shorter gradients to allow for processing more samples per day, the separations have become less reliable. As a result, there is more emphasis now on picking the ionization conditions to allow the compounds of interest to ionize more readily.

To get around these problems, LCMS method development often requires thinking the problem through backwards from the normal way of approaching HPLC problems. That is, look at the detection situation BEFORE picking a column type or mobile phase! Pick the source, the mobile phase and buffer to allow for sufficient sensitivity for the MS analysis. If the compound requires a particular pH or additive in order to ionize sufficiently, then this needs to be considered before selecting the HPLC column to use.

Priority in Developing LCMS Analytical Methods

3 Factors of Analytical Conditions
(4 if pretreatment conditions are included):
Detection > Separation > Injection

1. Quantitation most important
Detection > Separation > Injection

2. Qualitative info most important
Separation > Detection > Injection

General Approach to LC/MS Methods

- Pick the source for ionization (i.e., APCI or ESI) based on knowledge of the molecule and results required
- Pick the mobile phase that gives the best sensitivity (s/n)
- Use an additive (or buffer) only if necessary to improve the sensitivity and keep concentration as low as possible
- Pick a column that will provide the separation, resolution and peak shape required using the mobile phase and additive conditions already selected above
- Pick a standard based on separation from target, ability to ionize in similar fashion, and based on having a clean blank from the matrix at that mass

Keeping Ions Ionized

Once you go to all the trouble to get the chemistry right to create ions, you have only part of the job done.

It is still necessary to keep the ions ionized! There are several things that can contribute to loss of signal and nearly everyone runs into these at some point. The first is concentration effects. The second is ion suppression whereby an ion can lose charge to some other molecule such as the mobile phase itself, or some contaminant from the sample matrix. For example, in APCI, you start off by ionizing a mobile phase molecule. This evaporates and performs a charge transfer to your target molecule. But there is nothing that says a molecule has to keep that charge forever. In fact, molecules don't like to hold onto a charge and will give up charge to other molecules quite easily, especially when the molecules nearby have a higher proton affinity than the target. If any molecule happens close enough and if it has a greater proton affinity than your target, you can lose a charge.

To minimize ion suppression, keep sample amounts low, keep additive levels very dilute, and reduce the amount of matrix material that gets into the spray chamber.

Reduce contaminants

With HPLC, most methods will be designed to separate the target molecules from sample contaminants. Sometimes it is necessary to do some sample cleanup or solid phase extraction to eliminate interferences. With LC/MS methods, this can be even more of a requirement.

Although the mass differentiation allows for deconvolution of overlapping peaks, it may still be necessary to resolve these components chromatographically in order to prevent ion suppression or adduct formation. Even if only two species are present and the target has a much higher proton affinity than the contaminant, it is still likely that the contaminant can influence the results in an undesirable way. For example, in electrospray, ions will migrate to the surface of evaporating droplets in the spray process. As ions get to the surface, the buildup of surface charge will cause these to be expelled from the droplet. If there is an abundance of one species of ion relative to the target, it will tend to block the ion evaporation, and reduce the amount of ions detected.

At some point in time, every LCMS user is going to discover polyols as contaminants in a sample. These ionize by ESI and APCI, and are present in a number of places. One source of these is from reverse osmosis membranes. Glycols or glycerol are often used to keep membranes soft during shipping and handling. When installed, they don't change the 'megohms' of the water, and generally don't show up in UV detectors. But they can wreck havoc in an LC/MS system. Make sure you run a sufficient amount of water through a new membrane before using the water for LC/MS detection.

Another class of common impurities that gets found every day is polymer additives. These chemicals are found just about everywhere and are difficult to remove. It is quite common to find antioxidants such as hindered phenols, or slip agents such as erucamide or stearamide contaminating LC/MS spectra. To combat these, the best recourse is to minimize the amount of plastic that your samples, solvents and syringes will see.

One final suggestion, when you clean your mass spectrometer components, such as optical components or skimmers, make sure that you don't re-contaminate them after cleaning. Allowing the critical mass spectrometer parts to touch anything will contaminate them. Use only clean glass or metal surfaces to sit parts on when performing any cleaning or decontamination steps.

Summary

With reasonable care, a LCMS system can operate for years, detect compounds reliably at extremely low levels, and give reproducible results that are as good or better than any other HPLC detector. The limit of performance is in your hands.

- LCMS sensitivity is not an instrument issue, but a technique and chemistry issue
- To gain the most from any instrument, look at the following:
  - What conditions give the best ionization?
  - What solvents and additives produce the most ions?
  - What HPLC conditions and column will work with the best ionization conditions, and additives for ionization?

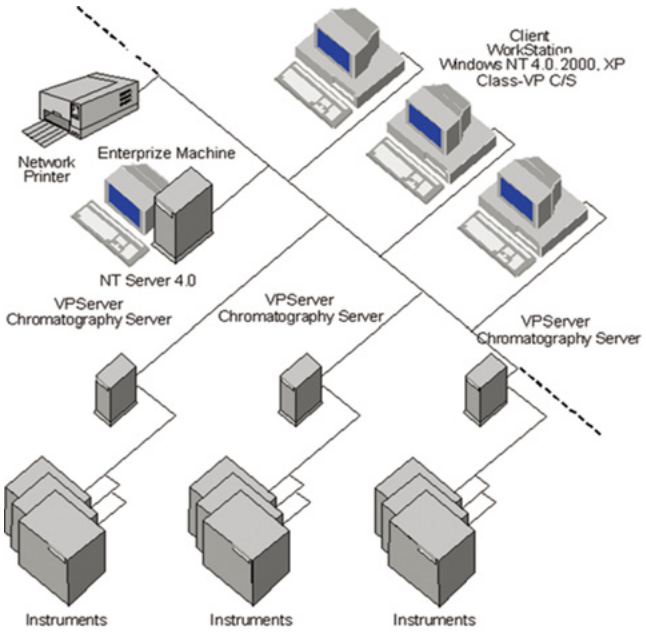
In past issues of LC WorldTalk, you have noticed the inclusion of LC Compliance Corner, which was designed to address FDA regulations such as 21 CFR Part 11, new trends in GxPs, risk analysis issues, validation of laboratory equipment and software, as well as new recommendations from GAMP and other topics. We have broadened the scope of this new section to encompass, in addition to regulatory compliance, other useful information on Shimadzu's Chromatography Instrument Control, Data Acquisition and Data Management Systems.

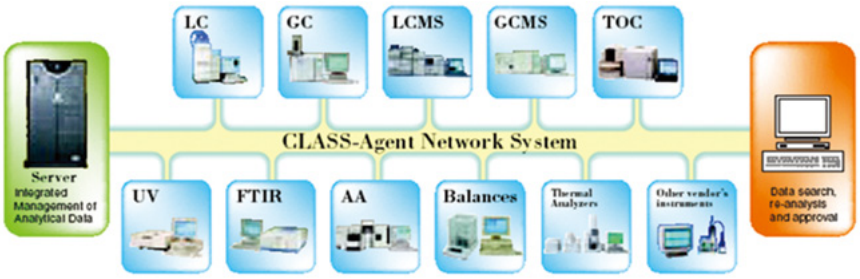
Shimadzu Chromatography and Data Management Software

In order to guarantee product quality and comply with regulatory requirements, efficient and reliable laboratory data systems are paramount. Shimadzu offers a wide range of chromatography and data management software products that can satisfy the needs of every laboratory.

CLASS-VP v7 Client/Server Chromatography Data System

CLASS-VP v7 is the latest version of our CLASS-VP chromatography software. This 32-bit package is ideal for C/S environments, so as to ensure full 21 CFR Part 11 compliance from validation to electronic signature to audit trail. CLASS-VP v7 allows the user to share information and control instrumentation from anywhere in the world in a completely secure environment. It is the complete solution for any laboratory chromatography data system requirement, providing maximum flexibility for demanding applications, including built-in spreadsheet and multiplexing options for multiple Shimadzu fraction collectors and autoinjectors. CLASS-VP supports a wide range of HPLC and GC systems, including the GC-2010/14/15/17, and LC-10, LC-10Avp and LC-20A HPLC systems.





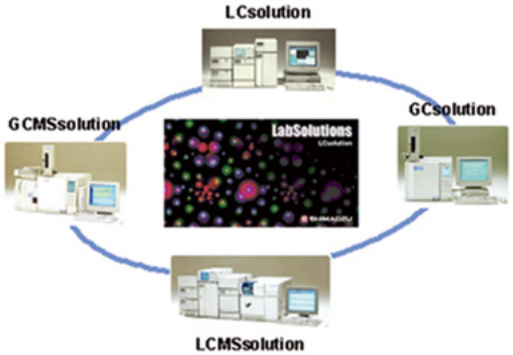
CLASS-CLASS–Agent Integrated Data Management Software

CLASS-Agent software provides a scalable solution to 21 CFR Part 11 compliance requirements for a wide range of laboratory instruments from AA and Balances to TOC and UV-Vis. It incorporates audit trail and electronic signature functions plus secure database archiving for Shimadzu Instruments' control software packages. CLASS-Agent provides users with a network-based browser for review and authorization of data packages. Easy-to-use functions allow users to preview chromatograms, create graphs and reports, and visually overlay multiple files for batch review. CLASS-Agent software seamlessly integrates with all Shimadzu data acquisition packages from CLASS-VP v7 to Lab Solutions, from TOC-Control V and WizAArd to UVProbe, IRsolution and Balance software. CLASS-Agent can also be used to archive records generated by some non-Shimadzu data acquisition packages.

Lab Solutions Software

- GCsolution Software
- GCMSsolution Software
- LCsolution Software
- LCMSsolution Software

Encompassing GC/MS, GC, LC, and LC/MS control software packages, powerful, easy-to-use Lab Solutions software can enable users to maximize the full potential of their system quickly and efficiently. All setup and quantitative functions can be performed on one screen and Wizards lead the user through the process. It allows customized applications under software control and provides universal reports for QA/QC verification and GLP/GMP compliance automatically. The system administration includes user administration, system policy setting, log browser, and audit trails, which support 21 CFR Part 11 compliance. Lab Solutions software enables programmable scan and SIM modes and provides full qualitative and quantitative support. Reports can be customized to any format with no macros required.



Shimadzu Support

All Shimadzu network system products incorporate functions for the Part 11 compliance and computer validation functions required by GxP. Shimadzu provides documentation, including IQ/OQ, Certificates of Compliance, and Inspection Test Result Reports based on Shimadzu ISO 9001 certified systems. Shimadzu's accredited service personnel offer full support for validation of a customer's Shimadzu products. Shimadzu provides comprehensive customer support for FDA compliance, including supplying the latest information on FDA regulations through seminars and workshops, participating in vendor audits demanded by the Agency, and actively assisting customers in complying with new FDA regulations.

Shimadzu HPLC, GC, Mass Spectrometers, UV-VIS spectrophotometers and other spectroscopy products and their associated data processing systems all incorporate sophisticated, leading-edge technology for audit trail, security, and integrity functions to comply with cGMP and GLP demands. In addition to offering instruments and network-compatible software products, Shimadzu offers support for creating system operation and management procedures, provides information, organizes seminars, and offers post-installation training on Part 11.

The FDA does not, and cannot, certify the hardware, software or

# of Part 11 Compliance

services of specific analytical instrument and software manufacturers as Part 11-compliant. The reason being that compliance with the regulations requires management and operating procedures and the associated documentation for the system, which involves operational requirements additional to the functions offered by the product. Consequently, the creation of the company policy and validation master plan by the customer is extremely important for Part 11 compliance.

Therefore, Shimadzu offers the user meticulous support over the entire lifecycle of the product, from consultations before installing a new system to regular post-installation inspections. Shimadzu's in-house systems remain alert to the new requirements of regulatory agencies and national and international trends to continue to offer comprehensive support for customer requirements.

More importantly, Shimadzu routinely subjects its instrument control, data acquisition and archiving software packages to 3rd-party audits to obtain an additional level of assurance that the software can support the most stringent compliance needs. In October 2003, Dr. Sandy Weinberg, industry-recognized compliance expert, visited Shimadzu's US headquarters in Columbia, Maryland to perform a thorough evaluation of Shimadzu data systems.

Dr. Sandy Weinberg holds a Ph.D. from the University of Michigan and a Post-Doctoral degree from the University of Florida. His career activities span both the biological science industry and the business development arena, working with the FDA and other regulatory agencies to speed the development and control of the regulation of biologicals. Currently, Dr. Weinberg serves as Senior Director of Fast Trak Vaccines, a GE Healthcare initiative (formerly Amersham Biosciences), where he is responsible for helping to speed the development of vaccines for biodefense and other health issues.

Dr. Weinberg is the author of sixteen books on biomedical regulation and validation. He is also the author of two newsletters ("Early Warning" and "Advanced Notice"), and has written and presented more than 100 articles and speeches on cost-effective compliance. Dr. Weinberg is a regular columnist for American Biotechnology Laboratory, LIMSource, and The Journal of Bioprocessing. He has served as a consultant to the USFDA, USEPA, U.S. Surgeon General, Netherlands RIVM, Canadian Bureau of Biologics, Swiss Cantonical Ministry of Health, South African Ministry of Health, and a number of other international regulatory agencies and industrial organizations.

All Shimadzu data systems were audited as standalone and in the CLASS-Agent client/server environment. The purpose of this evaluation was to determine compliance adherence and functionality based on a point-by-point checklist derived from the current GAMP4 guidelines, emergent industry standard practices and the following US FDA regulations:

21 CFR Part 11 – Electronic Records, Electronic Signatures

21 CFR Part 50 – Protection of Human Subjects (also knows as Good Clinical Practice, GCP)

21 CFR Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies (GLP)

21 CFR Part 210 – Current Good Manufacturing Practice in Manufacturing, Processing, Packing or Holding of Drugs (cGMP)

21 CFR Part 211 – Current Good Manufacturing Practice For Finished Pharmaceuticals (cGMP)

21 CFR Part 820 – Quality System Regulation (also knows as cGMP for Medical Devices)

This evaluation also included elevant provisions from these US EPA regulations:

EPA Directive 2185 – Good Automated Laboratory Practices

Cross Media Electronic Reporting Rule (CROMERR)

All systems were found to be comprehensively validated and fully compliant with the above regulations.

International Seminars

As part of its commitment to providing comprehensive support for regulatory compliance, Shimadzu routinely holds seminars, training courses and workshops for its customers in all parts of the globe.

Alex Mutin giving a lecture on management of electronic records to Chinese pharmaceutical companies gathered in Shijiazhuang

In spring of 2004, Alex Mutin, Shimadzu's compliance expert, and Tony Margetts, the chief editor of GAMP, toured several cities in China and conducted seminars that focused on current risk-based interpretation of 21 CFR Part 11, validation of laboratory systems, CLASS-Agent as well as Lab Solutions software.

The seminars were held in Zhuhai in the South Guangdong province; Shijiazhuang, capital of Hebei province 280 km southwest of Beijing, a center for the study of traditional Chinese medicine and a center of the pharmaceutical industry; and, Zibo, an important industrial base in Shandong Province in China. Zibo has a complete industrial system with 35 main categories, including petrochemical, pharmaceutical, building materials, metallurgy, textile, light industry, electronics, etc.

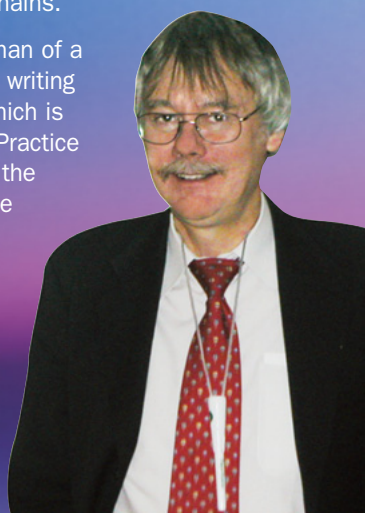
Each seminar focused on the current FDA position on Risk Analysis and Part 11. Special attention was given to the GAMP approach to validation of laboratory systems and procedures for handling electronic records and signatures. European and other regional regulations were also discussed. Lab Solutions and CLASS-Agent were introduced as a way to achieve an easy, efficient operation and ultimate compliance.

Speaker Profile

Dr. Tony Margetts has worked for AstraZeneca (formerly Zeneca and ICI Pharmaceuticals) since 1988. He has been responsible for a variety of international projects, e.g. leading teams responsible for technical transfers and preparations for FDA pre-approval inspections, and has also managed the introduction of new world-wide procedures and new products, including setting up supply chains.

During the 1990's, Dr. Margetts was the Chairman of a UK Pharmaceutical Industry Group charged with writing a Guideline on Computer Systems Validation, which is now called the Good Automated Manufacturing Practice (GAMP) Guide. Dr. Margetts is now chairman of the editorial board of the GAMP Forum and leads the editorial review of GAMP 4, which has recently been published. In addition, he led the Industry Group, which produced the recently published GAMP guideline on Electronic Records and Signatures.

He has recently retired from AstraZeneca and is now working as a consultant.



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